

Application No.: 09/155,252

Attorney Docket No.: SALK1470-2

Filing Date: September 21, 1998

(088802-1852)

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Amendments to the Claims

Please amend claims 27, 28, 36, 44 and 45 as indicated in the listing of claims below.

Listing of Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1.-15. (Cancelled).

16. (Previously presented) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR- γ , and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a hormone response element;

wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

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with the proviso that at least 4 nucleotides of said
-RGBNNM- sequence are identical with the nucleotides at
corresponding positions of the sequence -AGGTCA-; and
wherein said response element is optionally preceded by N_x, wherein x
falls in the range of 0 up to 5, and

- (c) a DNA segment encoding a reporter protein,
wherein said reporter protein-encoding DNA segment is operatively linked
to said promoter for transcription of said DNA segment, and
wherein said hormone response element is operatively linked to said
promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when said cells are
contacted with said compound, relative to the level of the reporter protein when said cells are not
contacted with said compound, is indicative of a compound that regulates the transcription-
activating effects of said receptor.

17. (Cancelled).

18. (Previously presented) A method according to claim 16 wherein said response
element has at least one copy of the minimal sequence:

AGGACA A AGGTCA (SEQ. ID NO. 5),

wherein said minimal sequence is optionally flanked by additional residues.

19. (Previously presented) A method according to claim 16 wherein said response
element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ. ID NO. 6).

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20. (Previously presented) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR- γ , and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a hormone response element, and
- (c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein said compound is a putative antagonist for said PPAR- γ , and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said PPAR- γ ,

wherein a decrease in the level of the reporter protein when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

21.-26. (Cancelled).

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27. (Currently amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with (i) a test said compound, and ~~in the further presence of (ii)~~ at least one additional compound that is a PPAR- γ agonist;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR- γ , and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a hormone response element, and
- (c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said test compound and said agonist, relative to the level of the reporter protein when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

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28. (Currently amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with (i) a test said compound, and ~~in the further presence of (ii)~~ at least one additional compound that is a PPAR- γ antagonist;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR- γ , and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a hormone response element, and
- (c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said test compound and said antagonist, relative to the level of the reporter protein when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

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29. (Withdrawn) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said cells express native PPAR- γ , and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a hormone response element, and
- (c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when said cells are contacted with said compound, relative to the level of the reporter protein when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

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30. (Withdrawn) A method according to claim 29, wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-; and

wherein said response element is optionally preceded by N_x, wherein x falls in the range of 0 up to 5.

31. (Withdrawn) A method according to claim 30, wherein said response element has at least one copy of the minimal sequence:

AGGACA A AGGTCA (SEQ. ID NO. 5),

wherein said minimal sequence is optionally flanked by additional residues.

32. (Withdrawn) A method according to claim 30, wherein said response element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ. ID NO. 6).

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33. (Withdrawn) A method according to claim 29, wherein said compound is a putative antagonist for said PPAR- γ , and wherein said contacting is carried out in the presence of increasing concentrations of said compound, and a fixed concentration of at least one agonist for said PPAR- γ ,

wherein a decrease in the level of the reporter protein when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

34. (Withdrawn) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR- γ agonist,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said compound and said agonist, relative to the level of the reporter protein when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

35. (Withdrawn) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR- γ antagonist,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

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36. (Currently amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing a GAL4 chimeric PPAR- γ receptor and a reporter vector with a test said compound;

wherein said GAL4 chimeric PPAR- γ receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding at least the ligand binding domain of a PPAR- γ and a DNA segment encoding a GAL4 DNA binding domain, and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a GAL4 response element capable of being bound by said GAL4 DNA binding domain, and
- (c) a DNA segment encoding a reporter protein,
wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and
wherein said GAL4 response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when said cells are contacted with said test compound, relative to the level of the reporter protein when said cells are not contacted with said test compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

37. (Previously presented) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain is introduced at the amino terminus of the DNA segment encoding said ligand binding domain of a PPAR- γ .

38. (Previously presented) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain is introduced at the carboxy terminus of the DNA segment encoding said ligand binding domain of a PPAR- γ .

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39. (Previously presented) A method according to claim 36, wherein the DNA segment encoding the native DNA binding domain of PPAR- γ is substituted with the DNA segment encoding said GAL4 DNA binding domain.

40. (Previously presented) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain encodes amino acid residues 1-147 of the GAL4 protein.

41. (Previously presented) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain encodes amino acid residues 1-90 of the GAL4 protein.

42. (Previously presented) A method according to claim 36, wherein the DNA segment encoding said GAL4 DNA binding domain encodes amino acid residues 1-74 of the GAL4 protein.

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43. (Previously presented) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing a GAL4 chimeric PPAR- γ receptor and a reporter vector with said compound;

wherein said GAL4 chimeric PPAR- γ receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding at least the ligand binding domain of a PPAR- γ and a DNA segment encoding a GAL4 DNA binding domain, and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a GAL4 response element capable of being bound by said GAL4 DNA binding domain, and
- (c) a DNA segment encoding a reporter protein,
wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and
wherein said GAL4 response element is operatively linked to said promoter for activation thereof,

wherein said compound is a putative antagonist for said PPAR- γ , and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said PPAR- γ ,

wherein a decrease in the level of the reporter protein when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

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44. (Currently amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing a GAL4 chimeric PPAR- γ receptor and a reporter vector with (i) a test said compound, and ~~in the further presence of (ii)~~ at least one additional compound that is a PPAR- γ agonist;

wherein said GAL4 chimeric PPAR- γ receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding at least the ligand binding domain of a PPAR- γ and a DNA segment encoding a GAL4 DNA binding domain, and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a GAL4 response element capable of being bound by said GAL4 DNA binding domain, and
- (c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said GAL4 response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said test compound and said agonist, relative to the level of the reporter protein when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

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45. (Currently amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing a GAL4 chimeric PPAR- γ receptor and a reporter vector with (i) a test said compound, and ~~in the further presence of (ii)~~ at least one additional compound that is a PPAR- γ antagonist;

wherein said GAL4 chimeric PPAR- γ receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding at least the ligand binding domain of a PPAR- γ and a DNA segment encoding a GAL4 DNA binding domain, and

wherein said reporter vector comprises:

- (a) a promoter that is operable in said cell,
- (b) a GAL4 response element capable of being bound by said GAL4 DNA binding domain, and
- (c) a DNA segment encoding a reporter protein,
wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and
wherein said GAL4 response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein when cells are contacted with said test compound and said antagonist, relative to the level of the reporter protein when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.